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Successful inoculation of *Artemia* and production of cysts in man-made salterns in the Philippines[1]

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Abstract

The objective of the inoculation described in this paper was to test the feasibility of culturing *Artemia* in man-made earthen salterns and of producing adults and cysts for use in aquaculture projects in the Philippines. San Francisco Bay (California, USA) *Artemia* were inoculated in two concrete tanks and in four earthen ponds which are part of a small local salt factory.

It was found that *Artemia* can be grown (with continuous production of nauplii and cysts) year-round in covered concrete tanks and in open concrete tanks and earthen ponds during the dry season (February to June). Lethal effects of too high water temperatures (> 35 °C) to the cultures were anticipated by the use of green coconut fronds placed on the water surface alongside the walls of the tanks and the earthen dikes.

Rice bran enriched with vitamins and traces of minerals appeared to be a good food for *Artemia* cultured in aerated concrete tanks ; in the earthen salt ponds the brine shrimp grew well on the natural food present.

Over a 3 month production period, 26 kg dry weight cysts and 150 kg live weight adults have been harvested from a total surface of 1.7 ha of salt ponds and brine tanks.

Introduction

It has been repeated at various occasions that the limited provision of *Artemia* cysts may lead to a serious bottle-neck in many aquaculture developments (Sorgeloos, 1979, 1980). The areas in the world where no natural populations of brine shrimp are occurring (Persoone and Sorgeloos, 1980) are most affected, since their aquaculture industry is entirely dependent on cyst import. This is particularly the case in Southeast-Asia where the aquaculture activities are

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increasing exponentially (Anonymous, 1979), *e.g.* "... the inadequate supply of brine shrimp for feeding shrimp larvae remains as the major constraint in the mass propagation of penaeids in Thailand as in the other countries" (ASEAN, 1977).

As explained in detail in a recent review paper on the ecology and biogeography of *Artemia* (Persoone and Sorgeloos, 1980), the pronounced climatic alternations of seasons : dry from November to April and wet during the rest of the year, most probably preclude the perennial establishment of brine shrimp in the Philippines.

The environmental conditions appear, however, to be suitable for *Artemia* during the dry season. Indeed thousands of hectares of saltponds are under operation. As a result, a man-managed inoculation program similar to the one accomplished successfully in the Macau (Rio Grande do Norte, Brazil) salt ponds (Sorgeloos, 1978 ; Sorgeloos *et al.*, 1979) could be considered in the Philippines. Contrary to Brazil where the new brine shrimp population is established permanently, the inoculation in Southeast-Asia will be of a temporary nature, *i.e.* the *Artemia* population will be eliminated by predation at the onset of the rainy reason. Pond production of brine shrimp in Southeast-Asia will thus be a cyclic operation (one cycle per year) and can eventually be integrated with salt production.

The experiments reported in the present paper describe the first *Artemia* inoculation trials in small saltponds in the Philippines.

The 1978 inoculation test and its results

The saltfarm where the inoculation was performed on February 22, 1978 is located in Barotac Nuevo (on Panay Island in the central part of the Province of Iloilo).

Two brine tanks in concrete ($15 \times 20 \times 0.75$ m) and several ponds with walls in concrete and earthen bottom of 3 400 m^2 surface each, were used for the *Artemia* production tests. Twenty million nauplii were hatched out of 100 g San Francisco Bay[6] (California, USA) cysts in the SEAFDEC-hatcheries at Tigbauan. Since it was only discovered at a later stage that freshly hatched nauplii can be transferred from natural seawater into brine of 100‰ and more, without their survival being affected (Sorgeloos, 1978), the nauplii were adjusted gradually to 90 ‰ seawater prior to their transfer to Barotac Nuevo.

The nauplii were transported in two 20 l plastic bags cooled to 4 °C and aerated with battery-operated aquarium pumps. Since the salt ponds were not ready for the inoculation experiment, the nauplii were distributed over two concrete tanks containing seawater of 84 and 130 ‰ salinity respectively.

The water in the tanks was aerated with the aid of 16 airstones connected to a 0.5 HP compressor. The animals were fed twice a week with a suspension of fine rice bran (prepared by squeezing 8 kg pre-soaked rice bran through a flour bag), 50 cc tiki-tiki[7] (a rice bran extract rich in factors of the vitamin B complex) and 10 g poultry vitamin-mineral mix Tra-Phos-D[8]. Rice bran was added till the turbidity of the water reached 20-25 cm (immersion of a graduated stick).

[6] San Francisco Bay Brand Cy, batch 288-2596.

[7] United Laboratories, Inc. of the Philippines.

[8] Interchem Philippines, Corp.

After 10 days the animals had reached the adult stage and half of the population was harvested with nets and inoculated in two salt ponds the salinity of which was about 100 ‰ and the average water depth 30 cm. The animals thrived well in the ponds without aeration and without any supplemental feed. Their natural food was composed of detritus (the salt ponds' intake water from a mangrove-creek) and phytoplankton, mainly diatoms belonging to the genera *Bacteriastrum, Chaetoceros*, and *Coscinodiscus*, and the bluegreens *Lyngbya, Oscillatoria*, and *Spirulina*.

Water temperature in the ponds ranged from 25 °C at night up to 33 °C during the day. One day the temperature rose to 37 °C, resulting in a mass kill of brine shrimp. About 65 kgs of *Artemia* biomass (wet weight) was collected and frozen for later use.

To avoid a repetition of a mass kill due to intense solar radiation and consequent high water temperatures, coconut fronds (branches) were laid in three parallel rows along the sides of the concrete dikes. They were kept at the water surface by bamboo stakes. No mortality was noticed even when water temperature rose as high as 39 C. It was observed that the animals stayed in the shade of the coconut fronds where the water temperature was 3 to 5 °C lower than in the open water.

Water from an estuary was let into the ponds every 12 to 15 days with the tidal rise to replenish water lost through evaporation and percolation.

When the salinity in the *Artemia* ponds dropped by more than 20 ‰ during water intake, solar salt was added in order to adjust the salinity level. It was furthermore found that the *Artemia* culture did better when the pond bottoms were stirred with bamboo rakes twice daily (7 am and 5 pm). This action probably resulted in a better food distribution.

As the *Artemia* population expanded, more salt ponds were inoculated. At the end of the dry season, a total pond area of 1.6 ha contained brine shrimp.

Over the 3 month production period the water temperature in the ponds ranged from a low 25 °C to a high 39 °C (average 34.5 °C). The salinity fluctuated between 80 and 170 ‰ (average 136 ‰) and the pH between 7.0 to 8.5.

In the concrete tanks 20% of the culture medium was exchanged once a month by drawing water from the bottom with a pump provided with a fine mesh filter at its suction end (to avoid losses of brine shrimp) and refilling with water from the mangrove creek. The ranges for the abiotic conditions in these concrete tanks approximated 25 to 33 °C respectively (average 31 °C), 84 to 180 ‰ (average 93 ‰) and a pH ranging from 7.5 to 8.0.

From the end of March onwards, cysts were produced in the tanks as well as in the ponds. Whereas the *Artemia* population expanded through ovoviviparity at the lower salinity levels, more cysts were produced when the salinity reached 130 ‰ and higher. Since the wind direction was constant the cysts always accumulated in the same corner of the ponds and tanks.

The daily harvest of the cysts produced in the salt ponds was facilitated and even maximized by the installation of a plastic barrier which prevented the cysts of reaching the irregular dike structure where the harvesting with scoop nets was very difficult.

Processing and drying of the cysts was done following Sorgeloos (1978) and Sorgeloos *et al.* (1978).

At the end of the dry season (June 15th) about 16 kg cysts (dry weight) and some 150 kg adults (live weight) had been harvested from the ponds and the tanks. At the onset of the dry

season, an unquantified biomass of *Artemia* adults was left over in the ponds when these were utilized as nursery ponds for milkfish and prawn fry. The remaining brine shrimp constituted an excellent food for the former species.

During the 1979 dry season the same procedure of *Artemia* production has been repeated in the Barotac Nuevo salt ponds with even better resuls. During the period March-May 1979 cyst prodution averaged 620 gram dry weight/ha/day.

Discussion

The production results obtained at Barotac Nuevo clearly demonstrate the technical feasibility of *Artemia* cyst production in man-made salterns in Southeast-Asia.

It is obvious, however, that the procedures applied so far, can be improved greatly with regard to inoculation procedures, strain selection, water intake regimes, harvesting, etc. It was observed for example in these trials that high water temperatures (exceeding 35 °C) are lethal to the inoculated San Francisco Bay *Artemia*. This corroborates the observations of Baker (1966) for the parental population in the salinas of the San Francisco Bay. For this reason, the water depth in the Barotac Nuevo salt ponds was always maintained at 25 to 30 cm. This might, however, considerably restrict the area where brine shrimp could be produced since most of the salt ponds in Southeast-Asia operate at water depths of maximum 15 cm. To solve this problem, specific geographical strains of brine shrimp could be selected or developed through progressive adaptation to resist water temperatures above 40 °C. An alternative might be a compromise with the salt producers to maintain a higher water depth in order to arrive at an integrated production of salt and *Artemia*.

In view of the recent findings that there are considerable variations from one *Artemia* strain to another with regard to some specific characteristics which limit their use in the aquaculture hatcheries (not at least their nutritional value) the brine shrimp (cysts and adults) produced have to be "evaluated".

From the biometrical analyses of Vanhaecke and Sorgeloos (1980) it appears that the 1978 Barotac Nuevo cysts do not significantly differ from the parental San Francisco Bay stock.

Trial runs on feeding Barotac Nuevo *Artemia* to milkfish fry and juveniles resulted in high survival rates and fast growth. Fed a monodiet of decapsulated cysts, *Chanos chanos* fry grew from 0.024 g to 5 g with 80 to 95% survival. This is a much better figure than the average 65% survival obtained for fry grown in nurseries on a diet of ricebran and bluegreens algae. The daily consumption of decapsulated cysts averaged 30 cysts for larvae weighing 0.024 g. Juveniles with an individual weight of 5 g were fed nauplii and adult *Artemia* and benthic bluegreen algae, and attained an average weight of 250 g per fish after 72 days culturing.

Finally it has been shown that application of the *Artemia* inoculation technique can lead to a substantial increase in the production output of fish and shrimp nursery ponds. About 1 week prior to stock the fry, *Artemia* nauplii are inoculated in the ponds. Through proper screening of the intake waters, the ponds must be kept free from brine shrimp predators (except maybe a few insect species like Corixidae or Notonectidae the predatory activity of which can, however, in a period of one week not interfere too much with the development of the *Artemia* population). By the time the fry are released into the ponds, the *Artemia* have grown into pre-adults which are an excellent diet for the released fry.

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